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Taxonomic and ecological profile of 'green tide' species of *Ulva* (Ulvales, Chlorophyta) in central Philippines

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Abstract

Ulva spp. are common in the intertidal zones of the Philippines, but, at certain times, could over-proliferate producing blooms or 'green tide' in some protected bays. In Mactan Island (Cebu), central Philippines, at least two species constitute the *Ulva* population, either as free-living or attached form. The one referred to in the literature as '*Ulva lactuca*' mainly consists of free-living population while the species referred to as *Ulva reticulata* consists mainly of attached population. Based on morphological and physiological characteristics, '*U. lactuca*' differs much from the descriptions of the species from its type locality in Europe in having a crumpled texture of blade, presence of tooth-like protuberances at the margins, thinner thallus (40–50 μm) and more pyrenoids per cell (two to four). The species referred to as '*U. lactuca*' in the Philippines therefore is a different species. Two morphotypes consisted the '*U. lactuca*' population from Mactan – a thick thallus and a thin thallus type. However, both morphotypes cultured under the same condition in the laboratory could transform into the same thin-thallus type observed in the field. 'Green tide' caused by '*U. lactuca*' occur almost regularly in Station 1 of Mactan Island, reaching an average biomass of up to 2.6 kg wet wt m^{-2} (or 0.5 kg dry wt m^{-2}). *Ulva reticulata*, although was less abundant in the rocky tidal zone at most times, reaching an average biomass of only up to 0.15 kg wet wt m^{-2} (or 0.03 kg dry wt m^{-2}) had caused green tide in Station 2 around February–March. Reproductive structures were not observed in both *Ulva* species during the survey period suggesting that vegetative fragmentation is the main mode of propagation. Vegetative tissues excised from the thallus can be induced to release biflagellated large and small zooids.

Introduction

Species of the green algal genus *Ulva* (Ulvaceae, Chlorophyta) have been used since the 1970s in some countries as biofilters to remove nitrogen and phosphorus pollutants–chemicals which are often found in domestic wastewater and wastes from aquaculture facilities (Cohen & Neori, 1991; Neori et al., 1991; Jimenez del Rio et al., 1996). Thus, *Ulva* has been tagged as 'pollution indicator' due to its biomass accumulation in highly polluted waters (Morand et al., 1991). In the island of Cebu, in central Philippines, 'green tide' or algal proliferation caused by this green algal genus, is becoming a common phenomenon especially in areas associated with dense human population.

Based on the taxonomic guides of Trono & Ganzon-Fortes (1988) and Calumpong & Meñez (1997), *Ulva* in the Philippines consists of two species: '*U. lactuca*' and *U. reticulata* (Forsskål. The former is described as having 'thin, glossy broad sheets with lobed undulating margin' and the latter as having 'highly perforated thallus'. Free-living thalli of species identified as '*U. lactuca*' in the Philippines has, at least, two different morphotypes observed in Mactan Island. These morphotypes could be ecolo-

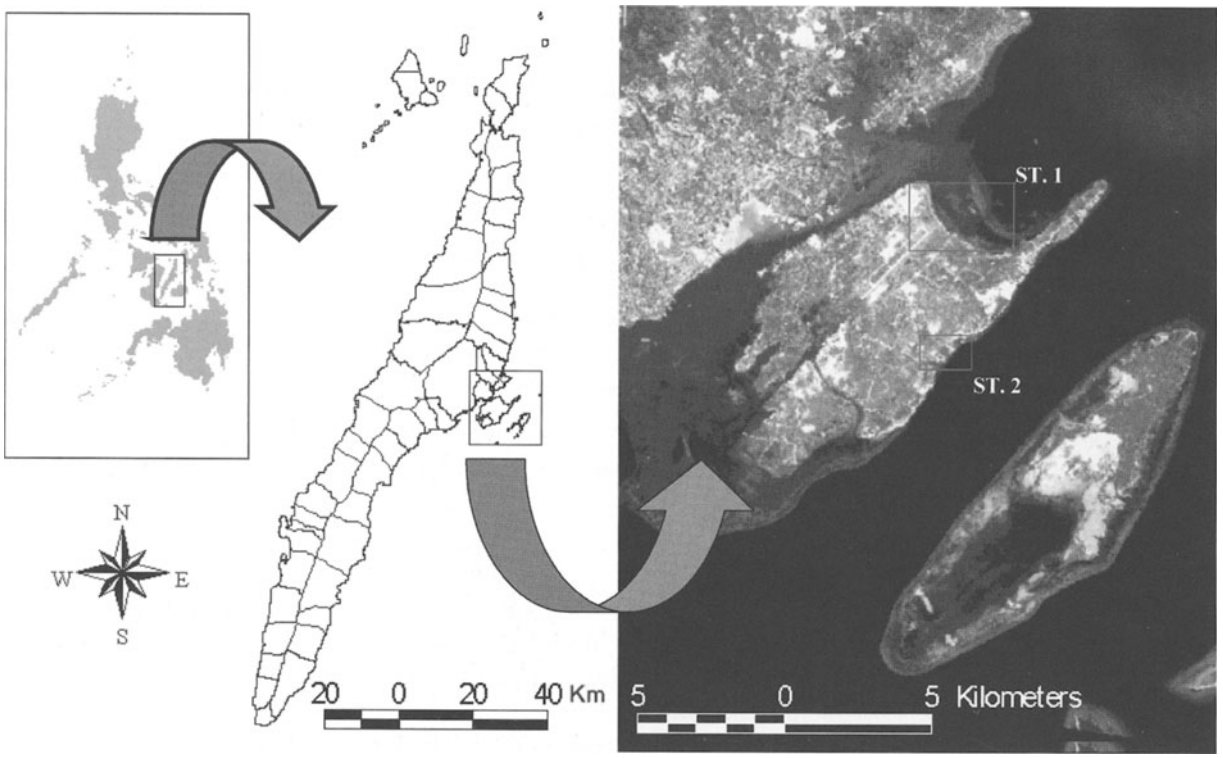


Figure 1. Map of the study area where the two monitoring stations of *Ulva* were established.

gical variants of the same species or could be entirely different species. The purpose of this study, therefore, is to provide baseline taxonomic and ecological data of *Ulva* in the Philippines and to document the occurrence of 'green tide' particularly in the increasingly industrialized island of Mactan (Cebu), in central Philippines.

Materials and methods

Description of the monitoring stations

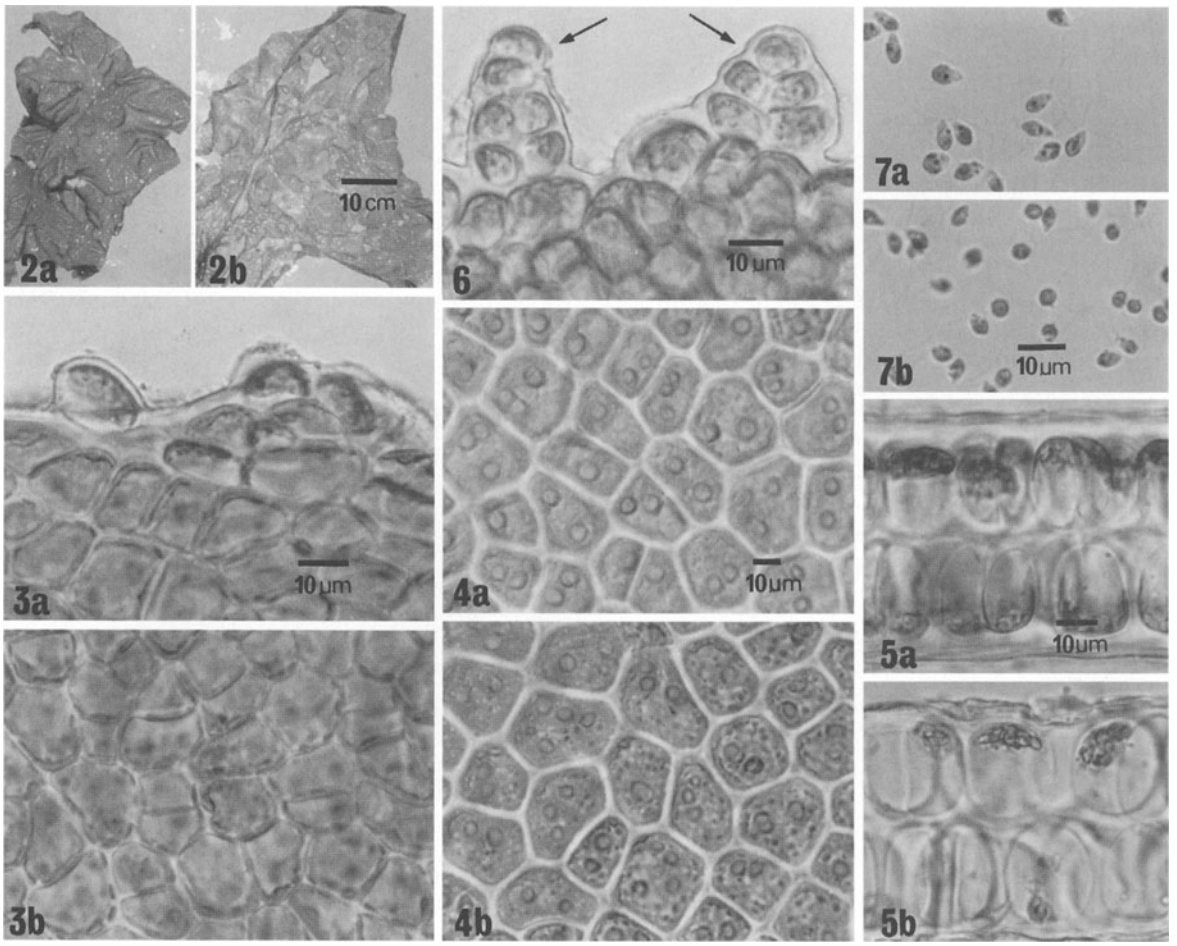
The monitoring of *Ulva* was conducted from December 1998 to January–July 1999 mainly in the intertidal areas of Mactan Island, Cebu. Monthly biomass of '*U. lactuca*' and *U. reticulata* was obtained at two stations within Mactan Island. Station 1 was established within Magellan Bay, in the northeastern side of the island (across the foreshore light guides of the Cebu International Airport runway) while Station 2 was located in the mid-eastern portion of Mactan Island (Fig. 1).

Morphological and reproductive examination

Fresh materials of both species from the field were obtained monthly for morphological and reproductive examinations. However, this study gave more attention to '*U. lactuca*' due to its taxonomic uncertainty. Cell size and shape in both surface and transverse views were determined; the number of pyrenoids and chloroplast concentration of the two morphotypes were noted as well. To induce zooid (spore/gamete) release, thalli pieces, approximately 3–4 mm diam., were excised by punching on vegetative thalli using the method described in Hiraoka & Enomoto (1998).

Measurements of biomass and conditions of surrounding waters

Biomass estimates were obtained monthly from 10 randomly placed 0.25-m^2 quadrats. Samples were oven-dried at 60 °C for 48 h. For convenience, *Ulva* was segregated into two morphologically distinct species as '*U. lactuca*' and *U. reticulata*, based on their general description by Trono & Ganzon-Fortes (1988) and by Calumpong & Meñez (1997). As part of a general description of the study area, water temper-



Figures 2–7. Morphological characteristics of '*Ulva lactuca*'. (2) Two morphotypes observed in the study area consisted of thick (2a) and thin (2b) thalli. (3) Surface view of thallus showing quadrangular marginal cells (3a) and polygonal middle cells (3b). (4) Surface view of thallus showing distinct pyrenoids ranging in number from mostly one to three per cell (4a) to mostly two to four per cell (4b). (5) Transverse section showing dense (5a) and sparse (5b) chloroplast concentrations. (6) Marginal tooth-like protuberances consisting of two-cell rows and an apical cell. (7) Release of large (7a) and small (7b) biflagellated zooids induced from excised tissues (by punching method).

ature and salinity were determined using fluid-filled thermometer and refractometer, respectively.

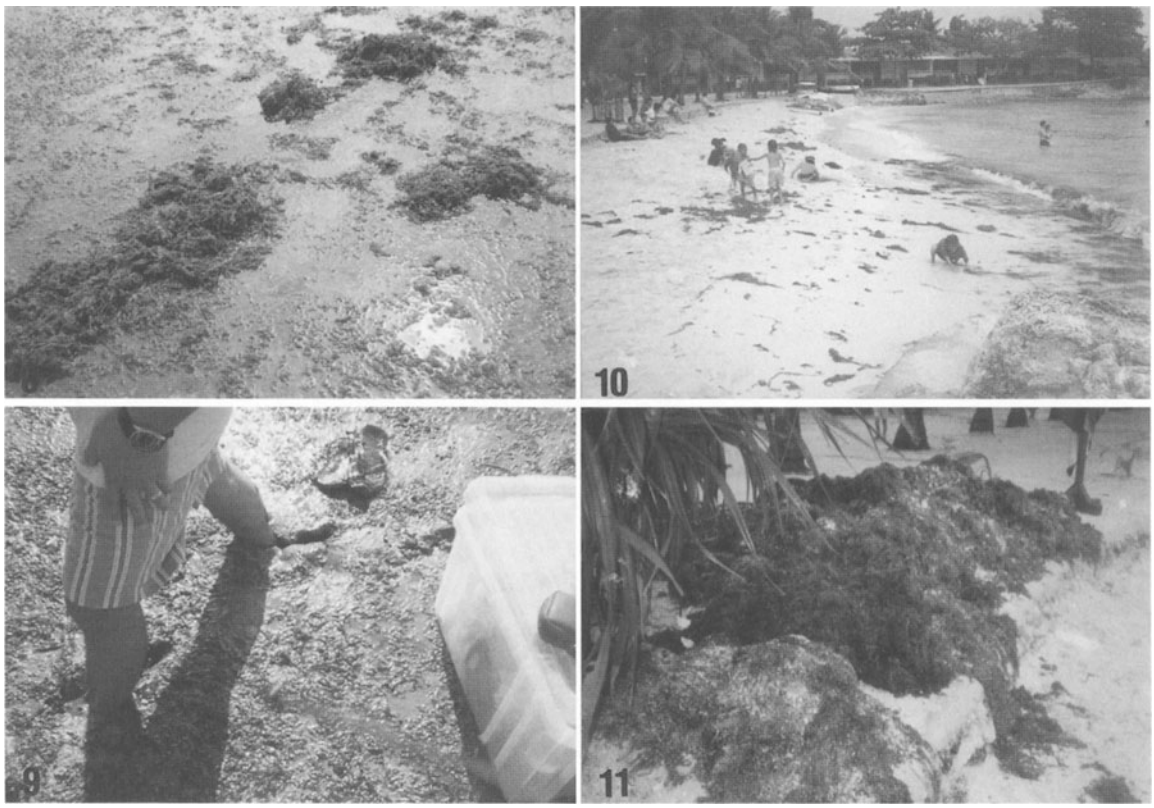
Growth rate measurements of 'U. lactuca'

The growth rate of '*U. lactuca*' was determined at different seawater temperatures – 18, 20, 22, 25, 28, and 30 °C, using a re-circulating culture system (Aquatron by Ohno, 1977). The increase in size of '*U. lactuca*' thalli was determined (in relative units) on a weekly basis using a computer image scan of 12 pressed samples obtained from the incubated materials. The growth rate based on 12 samples was computed as percentage increase per day using the formula described in Ohno et al. (1994). Except for temperature and weekly changes of seawater, condition for incubation was made constant at 100 µE m^{-2} s^{-1} (natural light+fluorescence light) at 12:12 h L/D photoperiod. Incubation period consisted of 7 days in each temperature regime.

Results and discussion

Ulva *morphotypes*

Ulva reticulata has highly perforated thallus in contrast to '*U. lactuca*' which has foliose thallus. However, the latter may also become perforated due to age. *Ulva* populations observed in Mactan Island were either free-living or attached, usually on hard substrate. *Ulva reticulata* mainly comprised the attached



Figures 8–11. (8) Massive accumulation of free-living '*Ulva lactuca*' during a 'green tide' event in the intertidal zone of Station 1 (northeastern side of Mactan Island). (9) *U. lactuca* green tide over 30–50 cm thick and dark mud layer characteristic of Station 1. (10) Green tide caused by *Ulva reticulata* occurring in a beach resort in Station 2 (mid-eastern side of Mactan Island) on February–March 1999. (11) Harvesting of *U. reticulata* biomass mixed with some other algal species heaped up on a beach resort during a green tide event in Station 2.

population of *Ulva* in the sandy-rocky substratum of Station 2 in Mactan Island. However, non-reticulate thalli of *Ulva* were also, but not often, observed in this station. '*U. lactuca*', on the other hand, almost exclusively dominated the free-living population found in the eutrophied waters of Station 1 and was observed to be of two morphotypes: thick (dark green; Fig. 2a) and thin (light green color; Fig. 2b). Transverse sections made from these thalli show variation in cell shapes and concentrations of chloroplast. Vegetative thalli of '*U. lactuca*' consisted of quadrangular, near the margin (Fig. 3a), to polygonal cells in midthallus (Fig. 3b). Pyrenoids were distinct in surface view, varying in number from 1 to 4 (often more in thick type; Fig. 4a,b). In transverse view, cells of thick-thallus type were generally elliptical while those of thin-thallus type were shorter and round. Chloroplasts also varied in concentration from dense (thick type; Fig. 5a) to sparse (thin type; Fig. 5b). When these two morphotypes were grown in similar culture conditions, their cell size and color became similar in character, revealing their morphological plasticity. Protuberances, consisting of one to two cells, marked the thallus margin (Fig. 6). The appearance of these marginal protrusions is strikingly similar to those in *Ulva armoricana* Dion, de Reviers *et* Coat described in Dion et al. (1998) paper. *Ulva lactuca* is cited by these authors (Dion et al., 1998) as having a north European distribution. Based on this account, and the difference in thallus characters of the Philippine morphotype with that of *U. lactuca* described from Europe (e.g., no tooth-like protuberances), it is suggested that species referred to as '*U. lactuca*' in the Philippines is a different species. While it is closely similar to *U. armoricana* in most features especially in having, at least, a crumpled texture of blade and presence of tooth-like protuberances (Table 1), a detailed eco-physiological and molecular-based taxonomic examination is necessary to unequivocably identify '*U. lactuca*' as entirely different species.

Table 1. Morphological characteristics of Philippine morphotype of '*Ulva lactuca*' with respect to European morphotypes of *U. lactuca* and *U. armoricana*, a closely similar species

Morphotypes	Author	Crumpled texture of blade	Tooth-like protuberances	Cell shape and size (μm)		Thickness in mid- and apical region	Pyrenoid number per cell
				In surface view (mid-section)	Transverse section (μm) (rhizoidal region)		
U. lactuca							
European type	Dion et al. (1998)	No	No	Mostly polygonal	Cylindrical	50–90	Mostly 1
Philippine type (Mactan, Cebu)	This study	Yes	Yes	Quadrangular to polygonal	Oval	40–50	1–4 (2–3 most)
U. armoricana	Dion et al. (1998)	Yes	Yes	Polygonal to quadrangular	Oval or spindle-shaped, with tapered ends	30–55 (80 in winter)	Mostly 1–2

Spore/gamete release induction

The materials of both *Ulva* species, as examined microscopically, were all vegetative during the study period, suggesting fragmentation to be the main mode of propagation in these species. Release of zooids in excised pieces was successfully induced in '*U. lactuca*' using the punching method described by Hiraoka & Enomoto (1998). Large (+) and small (−) biflagellate spore/gametes were released within 3 days from the excised tissues of '*U. lactuca*' by this method (Fig. 7).

Observations on Ulva *habitat*

The two monitoring sites were selected based on the contrast of their substrate and water conditions. The sampling stations were up to 1–1.5 m deep at high tide but bare, except in tide pools, during low tide. Water temperature in the intertidal zone ranged from 26.4 to 31 °C during high tide but may increase up to 38 °C in the shallow tide pools. In Station 1 (within Magellan Bay), 'green tide' caused by massive accumulation of '*U. lactuca*' in the intertidal zone was observed (Fig. 8). The waters in this area are highly eutrophied by sources from Cebu harbor and from a river fronting Magellan Bay (see map in Fig. 1). The layer of muddy sediment in this area could be as deep as 30–50 cm; Fig. 9). On more consolidated substrate, the upper mud layer is less than 30 cm which results to the poor algal diversity in this area. Confinement of the *U. 'lactuca'* population within the shallow zone could be a result of limited water circulation within the bay. Effluents from households and industrial firms discharged directly into rivers and streams could have exacerbated this bloom. Although no data is available regarding the extent of eutrophication of waters around Metro Cebu, based on analysis of water samples from 5 major rivers which practically empty into the surrounding waters, dissolved oxygen, BOD, coliform bacteria and heavy metal pollutants all indicated highly polluted waters (DENR 7, 1993). Owing to the potential of *Ulva* as a biofilter of nutrients, and possibly toxicants, such as heavy metals and other xenobiotic pollutants, the removal of *Ulva* biomass could be a possible remedy to reduce input of nutrients in this place.

In Station 2, located in the mid-eastern side of Mactan Island (fronting a beach resort) contrasted that of Station 1 in the northern side, in having still relatively pristine waters, this being far from the influence of the Cebu harbor and water run-off from mainland Cebu. This area is well-flushed by tidal current from a relatively open sea. *Ulva reticulata* predominated the month of January (during a northeast monsoon season) causing 'green tide' in the shallow waters of Station 2. The wave-cast materials heaped up on the beach in large amount causing nuisance to swimmers (Figs 10 and 11). The plants were mainly in their young vegetative stage.

Ulva *biomass*

The average biomass of *Ulva* was highest in March 1999. Biomass of '*U. lactuca*' in Station 1 ranged from 0.15 to 2.6 kg wet wt m^{-2} (0.02–0.45 kg dry wt m^{-2}; Fig. 12). As its growth declined to a low standing crop towards July, its blades became increasingly perforated and fragile. This was accompanied by the appearance of another green alga, *Enteromorpha intestinalis* (L.) Nees and occasional appearance of young *U. reticulata*.

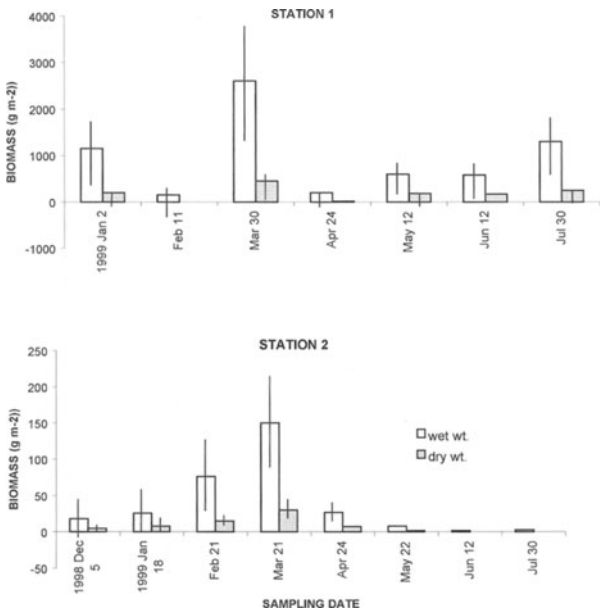


Figure 12. Monthly biomass of *Ulva* in green tide areas of Station 1 (above panel) and Station 2 (bottom panel) from December/January 1998/1999 to July 1999 as determined by quadrat sampling. *Ulva* population in Station 1 consisted almost exclusively of '*U. lactuca*' while Station 2 was predominated by '*U. reticulata*' (with few attached *Ulva lactuca)* all throughout the study period. Vertical lines represent standard deviation (n=10).

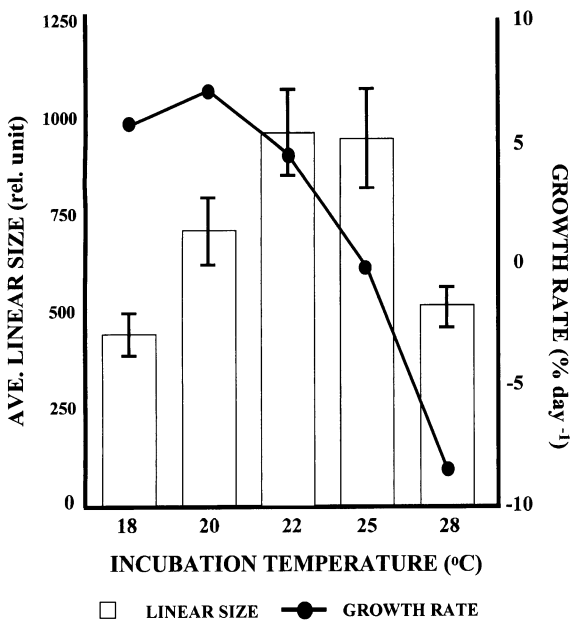


Figure 13. Relative size and growth rates of '*Ulva lactuca*' under different incubation temperatures in an aquatron culture system maintained at a light intensity of 100 μE m^{-2} s^{-1} under 12:12 h L/D photoperiod. Note maximum relative size and growth rate of this species at 20 and 22 °C, respectively. Incubation period was 7 days in each temperature regime. Vertical lines represent standard deviations (n=12).

In Station 2, the biomass of *U. reticulata*, which is a predominant species, increased dramatically from December to March where it attained its highest of 0.15 kg wet wt m^{-2} (0.03 kg dry wt m^{-2}; Fig. 12) during the study period. Highest values were recorded between February and March and were responsible for the unialgal bloom in this area during February–March (see Figs 10 and 11).

Growth rate of 'U. lactuca' *(no data for* U. reticulata*)*

'*U. lactuca*' grown in aquatron culture system at different temperature regimes showed highest growth rate of approximately 7% day^{-1} at 20 °C. However, highest increase in size was only observed at 22 °C (Fig. 13). Temperatures over 22 °C resulted in the decline of both growth rate and biomass under the aquatron condition. Highest biomass of '*U. lactuca*' and *U. reticulata* in the two stations was observed in February–March when *in situ* temperatures were generally low.

'*U. lactuca*' and *U. reticulata* showed distinct population dominance in two stations in Mactan (Cebu), Philippines suggesting both species to vary in their nutrient requirements, the former being more adopted to more eutrophied waters while the latter in low nutrient concentration. Future studies on *Ulva* in this area,

therefore, should be focused on its nutrient uptake variability.

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